

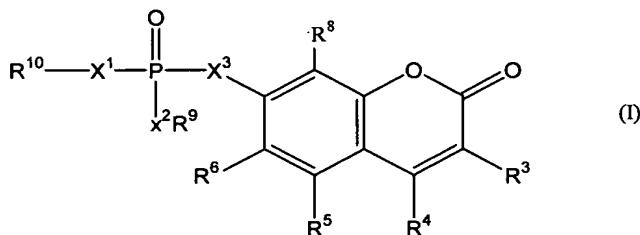
Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Please cancel claims 1-56 and add the following new claims:

57. (New) A compound of the formula I:



wherein

R³ is selected from the group consisting of H, cyano, C₁-C₆ alkyl, C₁-C₆ perfluoroalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, aryl, and heteroaryl, formyl, carboxamide of the formula -(C=O)NR¹R² where R¹ and R² are independently H, alkyl having 1-6 carbon atoms, an aryl, or R¹ and R² taken together form a saturated 5- or 6-membered ring having the formula -(CH₂)₂-M-(CH₂)₂- where the ring moiety M is a single bond, an oxygen atom, a methylene group, or the secondary amine -NR⁷- where R⁷ is H or alkyl having 1-6 carbon atoms;

R⁴ is selected from the group consisting of H, hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, sulfomethyl, salt of sulfomethyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, guanidino, C₁-C₆ alkylamino, C₁-C₆ acylamino, C₁-C₆ alkylamido, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₁-C₆ perfluoroalkyl, halomethyl, C₁-C₆ alkylthio, C₅-C₈ cycloalkyl, C₁-C₆ haloalkyl, C₅-C₈ halocycloalkyl, C₁-C₆ hydroxyalkyl, C₅-C₈ hydroxycycloalkyl, C₁-C₆ alkoxy C₁-C₆ alkyl, C₂-C₆ alkoxy carbonyl, C₂-C₆ alkoxy carbonyl C₁-C₆ alkyl, carboxy C₁-C₆ alkyl, carboxy C₁-C₆ alkoxy, dicarboxy C₁-C₆ alkyl, dicarboxy C₁-C₆ alkoxy, C₂-C₆ cyanoalkyl, phosphono C₁-C₆ alkyl, phosphoryl C₁-C₆ alkyl, mono-, di-, and trisaccharides, nucleic acids, oligonucleotides, amino acids, peptides, and proteins, and C₂-C₆ alkenyl, C₂-C₆ alkynyl, aryl, arylcarbonyl, and heteroaryl, which may be optionally substituted with a substituent selected from the group consisting of hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, and guanidine;

R⁵ is H or C₁-C₆ alkoxy;

R⁹ and R¹⁰ are ethyl;

R⁶ and R⁸ are halo; and

X¹, X², and X³ are independently O or S.

58. (New) The compound of claim 57, wherein R⁴ is selected from the group consisting of H, cyano, sulfomethyl, salt of sulfomethyl, aryl, C₁-C₆ alkyl, C₁-C₆ alkoxy, and C₁-C₆ perfluoroalkyl.

59. (New) The compound of claim 58, wherein R⁴ is selected from the group consisting of C₁-C₆ alkyl.

60. (New) The compound of claim 59, wherein R⁴ is methyl.

61. (New) The compound of claim 57, wherein R⁶ and R⁸ are fluoro.

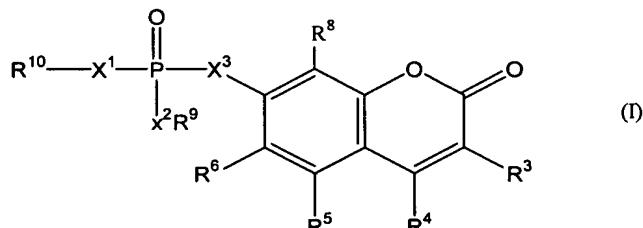
62. (New) The compound of claim 57, wherein R⁹ and R¹⁰ are ethyl, R⁴ is methyl, and R⁶ and R⁸ are fluoro.

63. (New) The compound of claim 57, wherein X¹, X², and X³ are O.

64. (New) The compound of claim 57, wherein X¹, X², and X³ are S.

65. (New) A method for specifically and selectively detecting and/or measuring the activity of an organophosphatase enzyme in a biological fluid, which contains at least organophosphatases and phosphatases, said method comprising:

(a) contacting the fluid with a compound of the formula I:



wherein

R³ is selected from the group consisting of H, cyano, C₁-C₆ alkyl, C₁-C₆ perfluoroalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, aryl, and heteroaryl, formyl, carboxamide of the formula -(C=O)NR¹R² where R¹ and R² are independently H, alkyl having 1-6 carbon atoms, an aryl, or R¹ and R² taken together form a saturated 5- or 6-membered ring having the formula -(CH₂)₂-M-(CH₂)₂- where the ring moiety M is a single bond, an oxygen atom, a methylene group, or the secondary amine -NR⁷- where R⁷ is H or alkyl having 1-6 carbon atoms;

R⁴ is selected from the group consisting of H, hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, sulfomethyl, salt of sulfomethyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, guanidino, C₁-C₆ alkylamino, C₁-C₆ acylamino, C₁-C₆ alkylamido, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₁-C₆ perfluoroalkyl, halomethyl, C₁-C₆ alkylthio, C₅-C₈ cycloalkyl, C₁-C₆ haloalkyl, C₅-C₈ halocycloalkyl, C₁-C₆ hydroxyalkyl, C₅-C₈ hydroxycycloalkyl, C₁-C₆ alkoxy C₁-C₆ alkyl, C₂-C₆ alkoxy carbonyl, C₂-C₆ alkoxy carbonyl C₁-C₆ alkyl, carboxy C₁-C₆ alkyl, carboxy C₁-C₆ alkoxy, dicarboxy C₁-C₆ alkyl, dicarboxy C₁-C₆ alkoxy, C₂-C₆ cyanoalkyl, phosphono C₁-C₆ alkyl, phosphoryl C₁-C₆ alkyl, mono-, di-, and trisaccharides, nucleic acids, oligonucleotides, amino acids, peptides, and proteins, and C₂-C₆ alkenyl, C₂-C₆ alkynyl, aryl, arylcarbonyl, and heteroaryl, which may be optionally substituted with a substituent selected from the group consisting of hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, and guanidine;

R⁵ is H or C₁-C₆ alkoxy;

R⁹ and R¹⁰ are ethyl;

R⁶ and R⁸ are halo or hydrogen; and

X¹, X², and X³ are independently O or S;

- (b) measuring the fluorescence of a fluorescent product formed during the contacting; and
- (c) correlating the measured fluorescence with the activity of the organophosphatase enzyme.

66. (New) The method of claim 65, wherein the organophosphatase is paraoxonase.

67. (New) The method of claim 65, wherein the organophosphatase is OPH.

68. (New) The method of claim 65, wherein R⁹ and R¹⁰ are ethyl, R⁴ is methyl, R⁶ and R⁸ are fluoro, and X¹, X², and X³ are O.

69. (New) The method of claim 65, wherein X¹ and X² are O, X³ is S, R⁶ and R⁸ are H; R⁹ and R¹⁰ are ethyl, and R⁴ is methyl.

70. (New) The method of claim 65, wherein the fluid is a biological fluid.

71. (New) The method of claim 70, wherein the biological fluid is selected from the group consisting of blood, blood-derived compositions, serum, cerebrospinal fluid, urine, saliva, milk, ductal fluid, tears, semen, cell or tissue extracts, culture medium from the expression of paraoxonase or mutations of paraoxonase, samples arising from the fractionation of paraoxonase or HDL from biological samples.

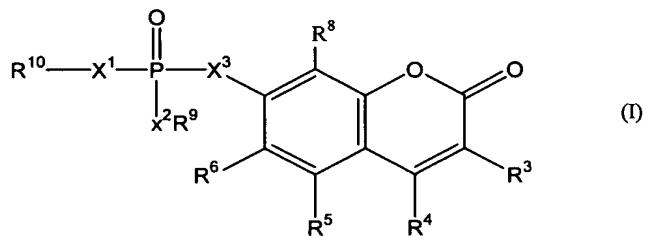
72. (New) The method of claim 71, wherein the cell or tissue extract is of brain, artery, vein or gland.

73. (New) The method of claim 70, wherein the fluid is an environmental fluid.

74. (New) The method of claim 73, wherein the environmental fluid is an extract of soil, water, or swab.

75. (New) A method for selectively detecting an organophosphatase in a sample suspected to contain an organophosphatase and a phosphatase comprising:

(a) contacting the sample with a compound of the formula I:



wherein

R³ is selected from the group consisting of H, cyano, C₁-C₆ alkyl, C₁-C₆ perfluoroalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, aryl, and heteroaryl, formyl, carboxamide of the formula -(C=O)NR¹R² where R¹ and R² are independently H, alkyl having 1-6 carbon atoms, an aryl, or R¹ and R² taken together form a saturated 5- or 6-

membered ring having the formula $-(\text{CH}_2)_2-\text{M}-(\text{CH}_2)_2-$ where the ring moiety M is a single bond, an oxygen atom, a methylene group, or the secondary amine $-\text{NR}^7-$ where R⁷ is H or alkyl having 1-6 carbon atoms;

R⁴ is selected from the group consisting of H, hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, sulfomethyl, salt of sulfomethyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, guanidino, C₁-C₆ alkylamino, C₁-C₆ acylamino, C₁-C₆ alkylamido, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₁-C₆ perfluoroalkyl, halomethyl, C₁-C₆ alkylthio, C₅-C₈ cycloalkyl, C₁-C₆ haloalkyl, C₅-C₈ halocycloalkyl, C₁-C₆ hydroxyalkyl, C₅-C₈ hydroxycycloalkyl, C₁-C₆ alkoxy C₁-C₆ alkyl, C₂-C₆ alkoxy carbonyl, C₂-C₆ alkoxy carbonyl C₁-C₆ alkyl, carboxy C₁-C₆ alkyl, carboxy C₁-C₆ alkoxy, dicarboxy C₁-C₆ alkyl, dicarboxy C₁-C₆ alkoxy, C₂-C₆ cyanoalkyl, phosphono C₁-C₆ alkyl, phosphoryl C₁-C₆ alkyl, mono-, di-, and trisaccharides, nucleic acids, oligonucleotides, amino acids, peptides, and proteins, and C₂-C₆ alkenyl, C₂-C₆ alkynyl, aryl, arylcarbonyl, and heteroaryl, which may be optionally substituted with a substituent selected from the group consisting of hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, and guanidine;

R⁵ is H or C₁-C₆ alkoxy;

R⁹ and R¹⁰ are ethyl;

R⁶ and R⁸ are halo or hydrogen; and

X¹, X², and X³ are independently O or S;

- (b) measuring the fluorescence of a fluorescent product formed during the contacting; and
- (c) correlating the measured fluorescence with the activity of the organophosphatase enzyme.

76. (New) The method of claim 75, wherein the organophosphatase is paraoxonase.

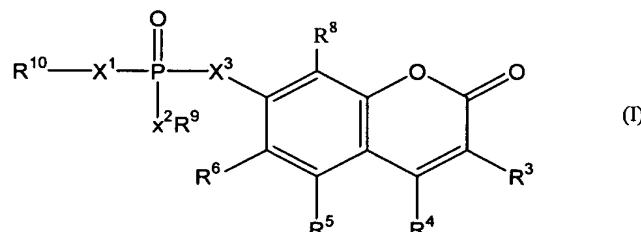
77. (New) The method of claim 75, wherein the organophosphatase is OPH.

78. (New) The method of claim 75, wherein R⁹ and R¹⁰ are ethyl, R⁴ is methyl, R⁶ and R⁸ are fluoro, and X¹, X², and X³ are O.

79. (New) The method of claim 75, wherein X¹ and X² are O, X³ is S, R⁶ and R⁸ are H; R⁹ and R¹⁰ are ethyl, and R⁴ is methyl.

80. (New) A method for specifically and selectively detecting and/or measuring the activity of an organophosphatase enzyme immobilized on a support, which comprises at least organophosphatases and phosphatases, said method comprising:

- (a) contacting the support with a compound of the formula I:



wherein

R³ is selected from the group consisting of H, cyano, C₁-C₆ alkyl, C₁-C₆ perfluoroalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, aryl, and heteroaryl, formyl, carboxamide of the formula -(C=O)NR¹R² where R¹ and R² are independently H, alkyl having 1-6 carbon atoms, an aryl, or R¹ and R² taken together form a saturated 5- or 6-membered ring having the formula -(CH₂)₂-M-(CH₂)₂- where the ring moiety M is a single bond, an oxygen atom, a methylene group, or the secondary amine -NR⁷- where R⁷ is H or alkyl having 1-6 carbon atoms;

R⁴ is selected from the group consisting of H, hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfanyl, sulfomethyl, salt of sulfomethyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, guanidino, C₁-C₆ alkylamino, C₁-C₆ acylamino, C₁-C₆ alkylamido, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₁-C₆ perfluoroalkyl, halomethyl, C₁-C₆ alkylthio, C₅-C₈ cycloalkyl, C₁-C₆ haloalkyl, C₅-C₈ halocycloalkyl, C₁-C₆ hydroxyalkyl, C₅-C₈ hydroxycycloalkyl, C₁-C₆ alkoxy C₁-C₆ alkyl, C₂-C₆ alkoxy carbonyl, C₂-C₆ alkoxy carbonyl C₁-C₆ alkyl, carboxy C₁-C₆ alkyl, carboxy C₁-C₆ alkoxy, dicarboxy C₁-C₆ alkyl, dicarboxy C₁-C₆ alkoxy, C₂-C₆ cyanoalkyl, phosphono C₁-C₆ alkyl, phosphoryl C₁-C₆ alkyl, mono-, di-, and trisaccharides, nucleic acids, oligonucleotides, amino acids, peptides, and proteins, and C₂-C₆ alkenyl, C₂-C₆ alkynyl, aryl, arylcarbonyl, and heteroaryl, which may be optionally substituted with a substituent selected from the group consisting of hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfanyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, and guanidine;

R⁵ is H or C₁-C₆ alkoxy;

R⁹ and R¹⁰ are ethyl;

R⁶ and R⁸ are halo or hydrogen; and

X¹, X², and X³ are independently O or S;

- (b) measuring the fluorescence of a fluorescent product formed during the contacting; and
- (c) correlating the measured fluorescence with the activity of the organophosphatase enzyme.

81. (New) The method of claim 80, wherein the organophosphatase is paraoxonase.

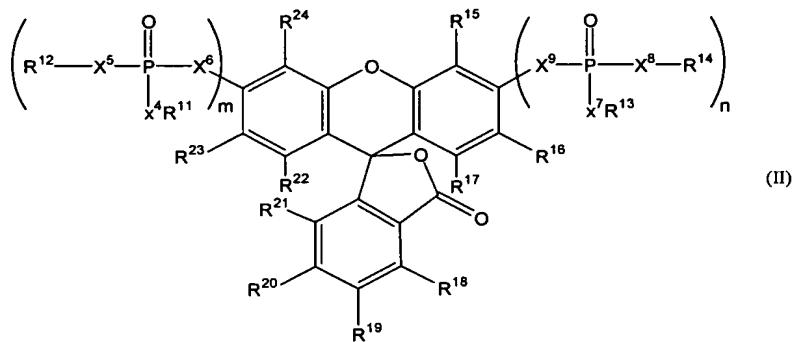
82. (New) The method of claim 80, wherein the organophosphatase is OPH.

83. (New) The method of claim 80, wherein the support is a membrane, resin, biosensor, microtiter plate, nanotube or dipstick.

84. (New) The method of claim 80, wherein R⁹ and R¹⁰ are ethyl, R⁴ is methyl, R⁶ and R⁸ are fluoro, and X¹, X², and X³ are O.

85. (New) The method of claim 80, wherein X¹ and X² are O, X³ is S, R⁶ and R⁸ are H; R⁹ and R¹⁰ are ethyl, and R⁴ is methyl.

86. (New) A compound of the formula II:



wherein

R^{11} - R^{14} are selected from the group consisting of C₁-C₆ alkyl, C₅-C₈ cycloalkyl, C₁-C₆ haloalkyl, C₁-C₆ perfluoroalkyl, C₂-C₆ alkenyl, and C₂-C₆ alkynyl, and aryl, arylcarbonyl, and heteroaryl, which may be optionally substituted with a substituent selected from the group consisting of hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, and guanidino;

X⁴-X⁹ are independently O or S;

n and m are 0 or 1 but m and n cannot be 0 simultaneously; and

R^{15} - R^{24} can be H or any substituent so long as the compound of formula II upon hydrolysis provides a fluorescent compound.

87. (New) The compound of claim 86, wherein the hydrolysis takes place at the P-X⁶ and/or P-X⁹ bonds.

88. (New) The compound of claim 86, wherein m and n are 1.

89. (New) The compound of claim 86, wherein R¹⁵-R²⁴ are independently selected from the group consisting of H, hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, sulfomethyl, a salt of sulfomethyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, guanidino, C₁-C₆ alkylamino, C₁-C₆ acylamino, C₁-C₆ alkylamido, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₁-C₆ alkylthio, C₅-C₈ cycloalkyl, C₁-C₆ haloalkyl, C₁-C₆ perfluoroalkyl, formyl, carboxamide of the formula -(C=O)NR¹R² where R¹ and R² are independently H, alkyl having 1-6 carbon atoms, an aryl, or R¹ and R² taken together form a saturated 5- or 6-membered ring having the formula -(CH₂)₂-M-(CH₂)₂- where the ring moiety M is a single bond, an oxygen atom, a methylene group, or the secondary amine -NR⁷- where R⁷ is H or alkyl having 1-6 carbon atoms, an aryl, or R¹ and R² taken together form a saturated 5- or 6-membered ring having the formula -(CH₂)₂-M-(CH₂)₂- where the ring moiety M is a single bond, an oxygen atom, a methylene group, or the secondary amine -NR⁷- where R⁷ is H or alkyl having 1-6 carbon atoms, C₅-C₈ halocycloalkyl, C₁-C₆ hydroxyalkyl, C₅-C₈ hydroxycycloalkyl, C₁-C₆ alkoxy C₁-C₆ alkyl, C₂-C₆ alkoxy carbonyl, C₂-C₆ alkoxy carbonyl C₁-C₆ alkyl, carboxy C₁-C₆ alkyl, carboxy C₁-C₆ alkoxy, dicarboxy C₁-C₆ alkyl, dicarboxy C₁-C₆ alkoxy, C₂-C₆ cyanoalkyl, phosphono C₁-C₆ alkyl, phosphoryl C₁-C₆ alkyl, mono-, di-, and trisaccharides, nucleic acids, oligonucleotides, amino acids, peptides, and proteins, and C₂-C₆ alkenyl, C₂-C₆ alkynyl, aryl, arylcarbonyl, and heteroaryl, which may be optionally substituted with a substituent selected from the group consisting of hydroxyl, cyano, nitro, halo,

amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, and guanidino.

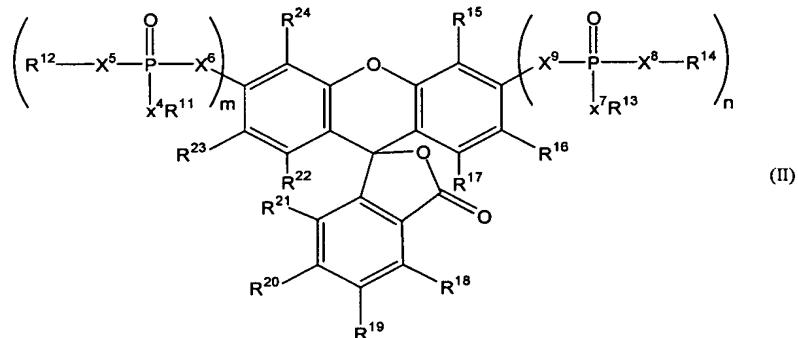
90. (New) The compound of claim 86, wherein R¹¹-R¹⁴ are independently selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, aryl, and heteroaryl.

91. (New) The compound of claim 86, wherein R¹¹-R¹⁴ are independently selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, and C₂-C₆ alkynyl.

92. (New) The compound of claim 86, wherein R¹¹-R¹⁴ groups are independently selected from the group consisting of C₁-C₆ alkyl.

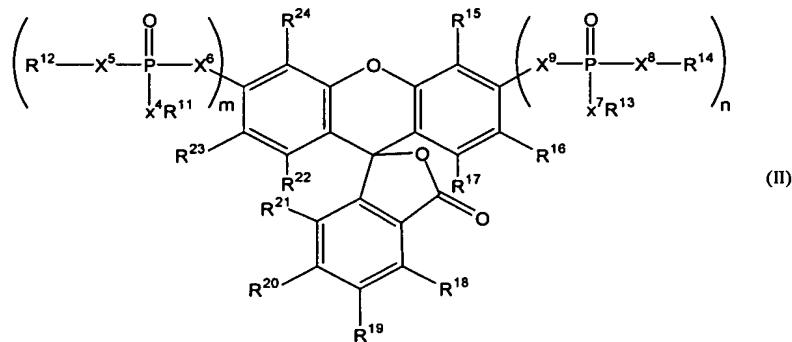
93. (New) The compound of claim 86, wherein R¹¹-R¹⁴ is ethyl.

94. (New) A compound of formula II



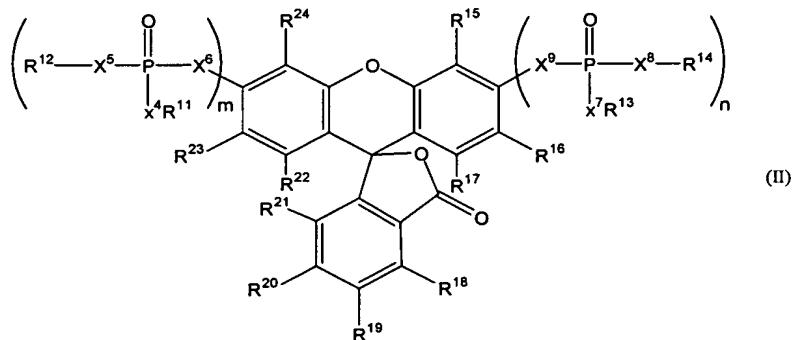
wherein X⁴-X⁹ are O, R¹⁵-R²⁴ are H, R¹¹-R¹⁴ are ethyl; and m and n are 1.

95. (New) A compound of formula II:



wherein X^4 , X^5 , X^7 , and X^8 are O; X^6 and X^9 are S; R^{15} - R^{24} are H; R^{11} - R^{14} are ethyl; and m and n are 1.

96. (New) A method for specifically and selectively detecting and/or measuring the activity of an organophosphatase enzyme in a fluid, which contains at least organophosphatases and phosphatases, said method comprising:



(a) contacting the fluid with a compound of the formula II;

wherein R^{11} - R^{14} are selected from the group consisting of H and groups or atoms other than H, X^4 - X^9 are independently O or S, n and m are 0 or 1 but m and n cannot be 0 simultaneously, and R^{15} - R^{24} can be H or any substituent so long as the compound of formula II upon hydrolysis provides a fluorescent product;

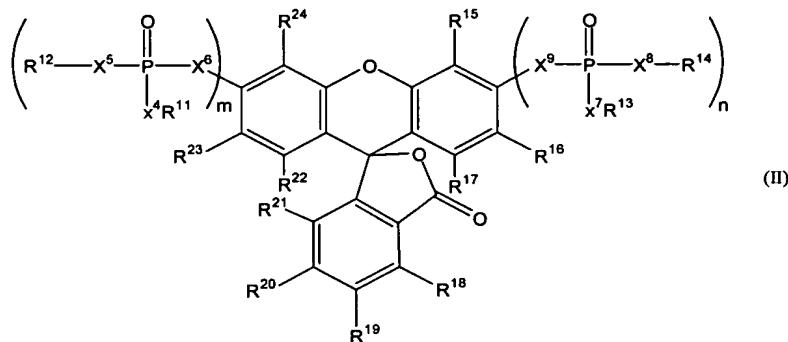
(b) collecting the fluorescent product;

(c) measuring the fluorescence of a fluorescent product formed during the contacting; and

- (d) correlating the measured fluorescence with the activity of the organophosphatase enzyme.

97. (New) A method for selectively detecting an organophosphatase enzyme in a sample suspected to contain an organophosphatase and a phosphatase comprising

- (a) contacting the sample with a compound of the formula II:

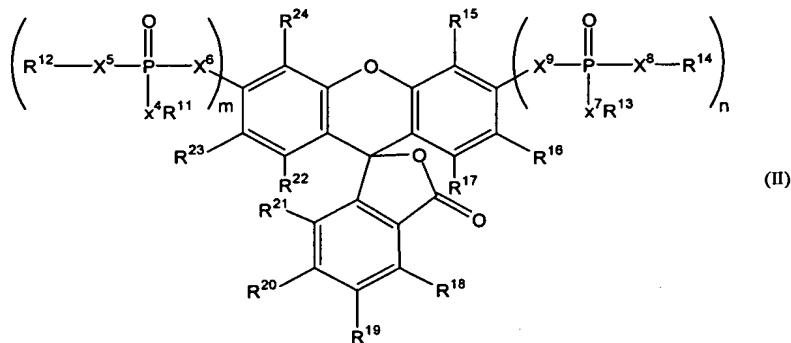


wherein R¹¹-R¹⁴ are selected from the group consisting of H and groups or atoms other than H, X⁴-X⁹ are independently O or S, n and m are 0 or 1 but m and n cannot be 0 simultaneously, and R¹⁵-R²⁴ can be H or any substituent so long as the compound of formula II upon hydrolysis provides a fluorescent product;

- (b) collecting the fluorescent product;
(c) measuring the fluorescence of a fluorescent product formed during the contacting; and
(d) correlating the measured fluorescence with the activity of the organophosphatase enzyme.

98. (New) A method for specifically and selectively detecting and/or measuring the activity of an organophosphatase enzyme immobilized on a support comprising:

- (a) contacting the support with a compound of the formula II:



wherein R¹¹-R¹⁴ are selected from the group consisting of H and groups or atoms other than H, X⁴-X⁹ are independently O or S, n and m are 0 or 1 but m and n cannot be 0 simultaneously, and R¹⁵-R²⁴ can be H or any substituent so long as the compound of formula II upon provides a fluorescent product;

- (b) collecting the fluorescent product;
- (c) measuring the fluorescence of a fluorescent product formed during the contacting; and
- (d) correlating the measured fluorescence with the activity of the organophosphatase enzyme.